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TRANSPORT PHYSIOLOGY

# Transgenic mice and their impact on kidney research

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**Abstract** The kidney is a key organ in the maintenance of ion and fluid homeostasis and specific transport systems localized along the nephron guarantee this function. Due to its large functional heterogeneity, experiments on the whole organ level cannot be easily performed, and thus more refined tools are needed, like for example the development of specific recombination systems to gain knowledge on the physiological role of single proteins implicated in ion transport. This review introduces the transgenic technology developed over the past decades, and then focuses on recent strategies for generating kidney-specific gene targeting, over-expression, and gene ablation in mice, that will help to understand the physiological role of proteins implicated in salt and water balance in the kidney.

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## Transgenic techniques

### Transgenic technology—an introduction

The genome alters during evolution or by specific breeding, a process that can be accelerated by inducing genetic modifications. This involves transgenic technology which allows to modify the genome (here, principally, of the mouse) at will and enables us to address specific scientific questions. Alternatively, germ cells of mice can be treated with mutagenic agents, as X-rays or ENU. The phenotype of the resulting mouse is scanned for specific defects, as exemplified by the recent efforts of the mouse clinic (GSF, Munich)[62]. Random alterations cannot compete for these directed modifications of the genome, such as classical transgenesis or knockout-related techniques. In the case of a transgene introduced by pronuclear injection, lentivirus or ES cell-mediated gene transfer, the insertion will be random, and is not linked to the endogenous locus if it exists. In contrast, knockout-related transgenesis will achieve a specific genetic modification at a given gene locus (Fig. 1, Table 1).

The history of transgenic mouse technology has existed for already several decades (Fig. 2). First transgenic experiments have been published 30 years ago, following infection of mouse embryos with viruses or retroviruses [41, 43]. Next, the generation of mice by microinjecting DNA into one of the pronuclei of a fertilized mouse oocyte [24, 110] still represents the most commonly used technique of generating transgenic mouse lines up to now and

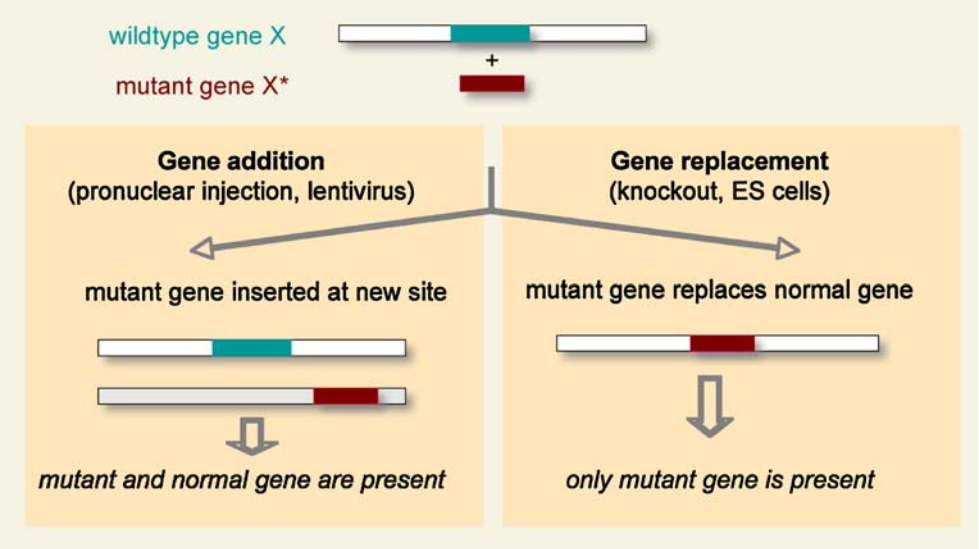


**Fig. 1** Transgenic mice can be obtained by adding new genetic information or by removing or mutating a gene already present in the genome

remained then nearly unchanged over the past three decades. Thousands of transgenic constructs have since been generated to answer a whole variety of scientific questions in biomedical research. Whereas earlier review articles could still follow the whole range of transgenic mice [42, 69, 90], nowadays, reviews focus on specific topics, as for example disease models or organ-specific transgenesis [39, 50]. Later in the 1980s of the past century, mouse embryonic stem cells (ES cells) isolated from the inner cell mass of preimplantation embryos (blastocyst stage) were established [18, 58] and first successful genetic manipulations of ES cells and generation of mice carrying the modification was reported more than 20 years ago [25]. A combination of gene-targeting approaches and these ES cells then allowed to generate the first knockout mouse, published in 1989 [93, 105]. The generation of such genetically engineered mice was honored in 2007 when the Nobel Prize for Medicine was attributed to Oliver Smithies, Mario Capecchi, and Martin Evans [56].

In the 1990s, a major breakthrough came up with the elaboration of the Cre/loxP-mediated recombination system [88], allowing to modify the genome either in the adult organism, or only in a specific tissue [45, 64]. First mice carrying so-called conditional alleles were published in the early 90s [26, 27], and, since then, the number of strains carrying conditional alleles or expressing Cre recombinase is steadily increasing. Only recently, another transgenic approach has emerged, using lentiviral vectors [55], that contributed to two major aspects, first allowing to manipulate species other than the mouse [94], and second, to silence gene expression in vivo using siRNA-expressing vectors [80]. Lentivirus-mediated transgenesis can be a very

**Table 1** A comparison of transgenic mice (pronuclear injection) and mice generated by homologous recombination (ES cells, knockout)

| | Transgenic mice (pronuclear injection) | Transgenic mice (ES cells and knockout) |
|---|---|---|
| Purpose | Gain-of-function | Loss-of-function, change-of-function |
| Studies | Overexpression | Knockout |
| | Define regulatory elements | A gene is absent or modified |
| | Disease model | Disease model |
| Technique | Injection of DNA into fertilized oocytes | Electroporation of DNA into ES cells |
| | | Injection of ES cells into blastocysts |
| | Transfer to oviduct of pseudopregnant female | Transfer to oviduct/uterus of pseudopregnant female |
| First mouse | Transgenic founder mouse (F0) | Chimera |
| Integration | Multiple copies | One copy |
| | Head-to-tail (tandem) | One integration site (independent integration sites, if several copies) |
| | Random | Random or directed |
| Construct | Minigene | Targeting construct |
| | Regulatory sequence–cDNA–polyA | 5′ homology–replacement–3′ homology |

Main differences between transgenic mice obtained by pronuclear injection (transgenic mice in the common sense) and homologous recombination in embryonic stem cells (knockout)

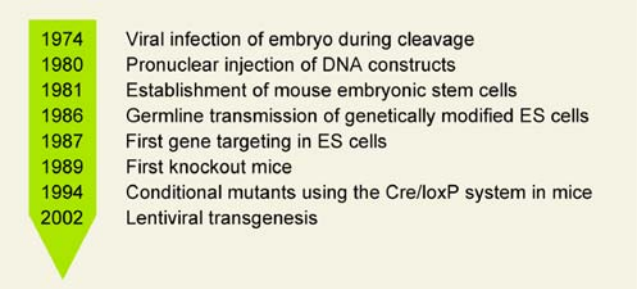


**Fig. 2** A timeline of transgenic technology, referring to several classical publications [10, 24, 26, 43, 55, 105, 110]

efficient way of generating transgenic mice. Due to the multiple independent integrations of the lentivirus, the establishment of stable lines can be tedious, but, nevertheless, the lentiviral approach represents a valid alternative when the effect of a transgene or mutant protein can be analyzed at the stage of the founder mice.

## Adding genetic information to the genome: pronuclear injection

In the following, we will detail the various aspects of gene "addition" in mice. Here, for ease of simplicity, we will focus on pronuclear injection which is the technique by far the most often applied today. ES-cell-mediated transgenesis will not be covered here, and specificities of lentiviral techniques are mainly related to the design of lentiviral vectors. Hence, the interested reader is referred to some recent articles and reviews for a more profound discussion [72, 80, 89, 101].

The various steps of the pronuclear injection technique are outlined in Fig. 3. Usually, female mice are superovulated with gonadotrophic hormones to synchronize the females and to obtain a larger number of maturing follicles per female. This can be done with almost any mouse strain, even though the efficiency is rather variable [65] and high doses of gonadotrophins may affect maturation and meiotic division in a certain percentage of oocytes [6]. F1 hybrid mouse strains are most often used, as for example B6D2F1 mice with the mother, B6 (C57BL/6), and the father, D2 (DBA/2). Alternatively, inbred strains as FVB/n or C57BL/6 might be used [65, 73, 102]. Fertilized oocytes (zygotes) are recovered when the paternal and maternal nucleus ("pronucleus") are not fused yet. DNA solution is injected into one of the pronuclei until visible swelling. For lentiviral transgenesis, the virus solution is injected below the glycoproteinic shell of the oocyte, the zona pellucida, which is less harmful for later survival. In both methods, surviving oocytes are then retransferred into the oviduct of pseudopregnant females. Such females have been obtained by mating with sterilized males (principally obtained by vasectomy), keeping them hormonally apt to accept a conception. If the procedure is successful, pregnancy of the mouse is visible after about 10–11 days, and mothers will give rise to birth after 19–20 days. Generally, the eventual transgenicity of the offspring is tested at weaning. The overall efficiency then depends, amongst other parameters, on the type of the construct, the quality of the DNA preparation, the choice of the mouse strain and the individual skill and performance of the experimenter.

The pronuclear injection technique allows the use of transgenic constructs of several 100 kb in size, using for example YACs (yeast artificial chromosomes), PACs (P1-derived artificial chromosomes) or BACs (bacterial artificial chromosomes) [23, 63, 103]. However, most often classical transgenic minigene constructs are used, which are up to 10–15 kb in length and are composed of a regulatory sequence which specifies the tissue of expression, a coding

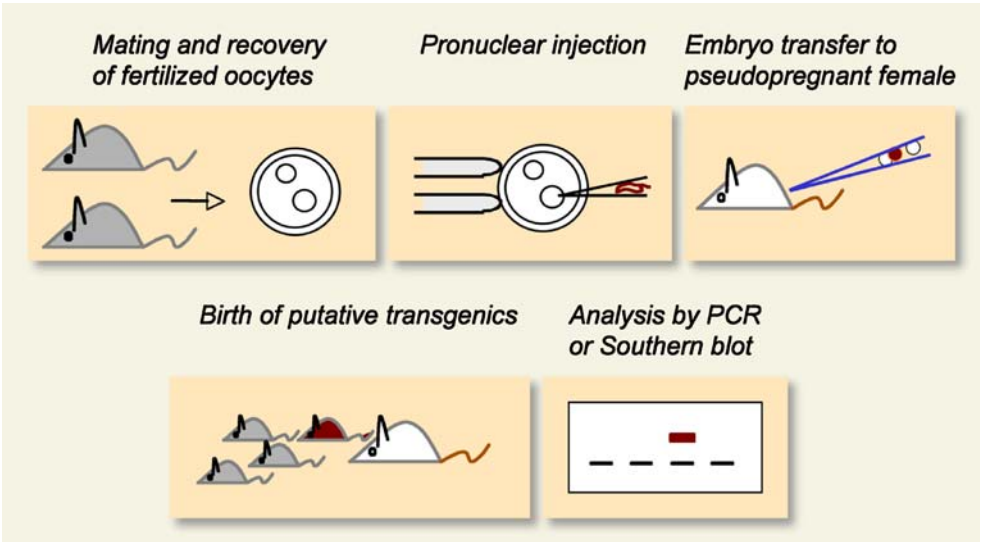


**Fig. 3** Scheme of the procedure for generation of transgenic mice by pronuclear injection

region (e.g., cDNA) and polyadenylation sequences. Often, intronic sequences are added to the design of the constructs, since this might enhance the stability of the transcript and increase the chance of expression [9]. Following removal of vector sequences, the concentration of the insert is calibrated to 1–5 ng/µl, and 1–2 pl of solution injected into one of the pronuclei.

Even though the injection of the DNA is effected during the one-cell stage (the fertilized oocyte), integration can occur later, for example in one cell of a four-cell stage. In consequence, the resulting transgenic mouse does not contain the transgene in all cells but only in a few of them—the mouse is mosaic for this transgene. As a consequence, the transgene may not be present in germ cells, and no transgenic offspring obtained. The integration of the transgene is random and multiple copies are normally integrated into a single site of the genome, but their chromosomal integration varies in independent transgenic mice. This integration normally occurs "head-to-tail" (tandem) in the same orientation, and encompasses mostly less than ten to 20 copies, even though higher copy numbers (up to 1,000) have been observed [54]. For example, when using a *SV40::c-Myc* transgene, more than 50 copies of the transgene were inserted on chromosome 8, whereas the endogenous *c-Myc* gene on chromosome 15 remained untouched [5]. A transgene is stably integrated and then inherited in a Mendelian fashion, and not lost over many generations [2]. Due to the randomness of integration, an endogenous gene may be affected, thereby rendering it unfunctional or modified in its expression. For example, a transgenic mouse line expressing the key enzyme in pigmentation, tyrosinase, showed skeletal malformations and polydactyly. It turned out that the transgenic insertion affected the *Gli3* gene, a member of the hedgehog signaling pathway, thereby generating a new allele at this locus [78].

## Embryonic stem cells and vectors

In contrast to the pronuclear, but also lentiviral transgenesis, ES (embryonic stem) cell-mediated approaches require two steps, first the genetic manipulation performed in cell culture, and second, the injection of such modified cells into mouse blastocysts that are 3.5-day-old-mouse embryos. Just before implantation, they can be recovered by flushing uteri [65, 73]. ES cells themselves are derived from the inner cell mass of mouse blastocysts, and need to be kept undifferentiated to maintain their stem cell characteristics. The leukemia inhibiting factor (Lif) known to be required to prevent differentiation of ES cells is either produced by irradiated primary mouse fibroblasts that are co-cultured with the ES cells and/or can also be added in its recombinant form to the culture medium. Several well-characterized ES cell lines are available which are most often derived from different substrains of 129 [97, 106] and are of male sex. Embryonic stem cells should exhibit unlimited self-renewal capacity but still maintain pluripotency. They can be amplified and manipulated similar to standard cell cultures in vitro, and it is feasible to select—following electroporation of gene constructs into for example $10^7$ cells—rare genetic modifications. Concordantly, when ES cells carrying a dominant allele of Agouti as $A^W$ (white-bellied agouti, from a substrain of 129) are injected into mouse blastocysts of the strain C57BL/6 (a, nonagouti), mice can result, which contain both C57BL/6- and 129-derived cells and are identified by coat color chimerism. ES cells then not only colonize somatic tissues of the blastocyst donor (C57BL/6), as evident from the coat color, but also the germ line. Following breeding of chimeras, the new modification can be fixed and bred as a new transgenic strain [65, 73].

Over the past two decades, various kinds of genetic modifications have been introduced in ES cells, ranging from random insertions (enhancer or gene trap approaches, [114]) and classical transgenic constructs [8, 25] to specific targeting of a sequence of interest (homologous recombination). In the mid 1980s, Mario Capecchi and coworkers showed that a gene defect in somatic cells can be rescued by introducing the correct sequence, which then recombines with flanking homologous sequences [104]. Consequently, this technique of homologous recombination allowed to generate mice in which a gene is deleted or specifically mutated [93, 105]. The classical knockout vectors used for constitutive gene inactivation contained a neomycin resistance gene to replace an endogenous exon—at the best to remove the ATG or any other vital region of the gene together with 5′ and 3′ homologous sequences of about 5 kb in total. Following electroporation into ES cells, selection markers and molecular analyses by PCR and Southern blot are required to identify the rare event of homologous recombination. Once identified, the ES cell clone is amplified, characterized and used for blastocyst injection to establish the mouse line [73]. As final outcome, a mouse line is generated where function of a specific gene is abolished or altered by replacement with a neomycin resistance gene. Using recombinase recognition sites in the targeting construct (loxP or frt, see below) the neomycin gene can be removed by further breeding to recombinase-expressing mice, to exclude any unwanted side effects. Nowadays, the technique of homologous recombination not only allows to generate null-mutations (classical knockout), but also subtle mutations (any kind of point mutations, microdeletions). Mouse models with chromosomal rearrangements (macrodeletions, inversions or translocations) as found in the human genome can be generated [11, 98]. Moreover, knock-in approaches allow not only to remove,

but to replace the gene by for example another member of the same gene family, to address issues of redundancy [57].

### Conditional knockouts

Since constitutive deficiency of a gene/protein may lead to embryonic or early perinatal lethality, conditional knockouts have been developed to study a mutation in a given cell type or in a timely controlled manner. The development of site-specific recombinases and recognition sites from bacteriophages (Cre/loxP-mediated recombination) or yeast (Flp/frt-mediated recombination) was a prerequisite for this technique (as depicted in Fig. 4). Two recombinase recognition sequences (34 bp loxP sites) are positioned in the same direction flanking a DNA sequence of interest (generally containing one or several exons). The Cre recombinase recognizes these loxP sites, induces a recombination which leads to excision of all DNA sequences between the two loxP sites, leaving one loxP site at this locus. The newer generation of knockout vectors possesses loxP sites 5′ and 3′ of a vital region, for example around the ATG-containing exon. In addition, the neomycin cassette is flanked by frt recognition sequences for Flp recombinase to eliminate the selection marker that may interfere with the expression of the modified gene. Following ES cell manipulations as detailed elsewhere [73] (see also above), a mouse is obtained which carries an exon flanked by two loxP sites and a neomycin cassette flanked by two frt sites. By mating this mouse with a transgenic strain expressing Flp recombinase in germ cells ("germ line deleter" [81]) the neomycin resistance gene is removed.

The Cre recombinase can be expressed in the germ cells or ubiquitously, thereby generating a constitutive knockout mouse, or in a cell type-specific and/or inducible manner [64]. Tissue-specificity is achieved by a tissue-specific promoter that targets Cre expression to the cell type or organ of interest. Consequently, the gene knockout is restricted to this cell type/organ. In its inducible version, Cre recombinase expression can be dependent on a doxycycline-controlled system [92], or alternatively, activity, and translocation of Cre recombinase to the nucleus is dependent on tamoxifen treatment [60]. This is due to a fusion of the Cre recombinase to the binding domain of the estrogen receptor (CreERT), and, upon administration of the synthetic hormone tamoxifen, Cre recombinase will enter the nucleus and exert Cre/loxP-mediated recombination. Functioning of the Cre/loxP-mediated recombination system can be tested by the use of reporter mouse strains that provide a mean to monitor specificity of Cre recombinase activity in vivo, e.g., using the Rosa26R strain [99]. Here, a STOP cassette flanked by two loxP sites and preceding a lacZ reporter gene was introduced at the ROSA26 locus. Cre activity results in removal of the STOP and permits lacZ expression which might be specific to the target tissue of interest thereby validating the tissue-specificity of the Cre transgenic line [30].

## Transgenes targeted to the kidney

### Kidney-specific transgenic expression

Genetic engineering in mice nowadays allows us to express transgenes specifically in the kidney, even though anatomic complexity of the kidney makes it difficult to select appropriate promoters that target a specific cell type, e.g., along the nephron. Even, a conditional approach is required (Fig. 4) when the constitutive knockout affects embryonic

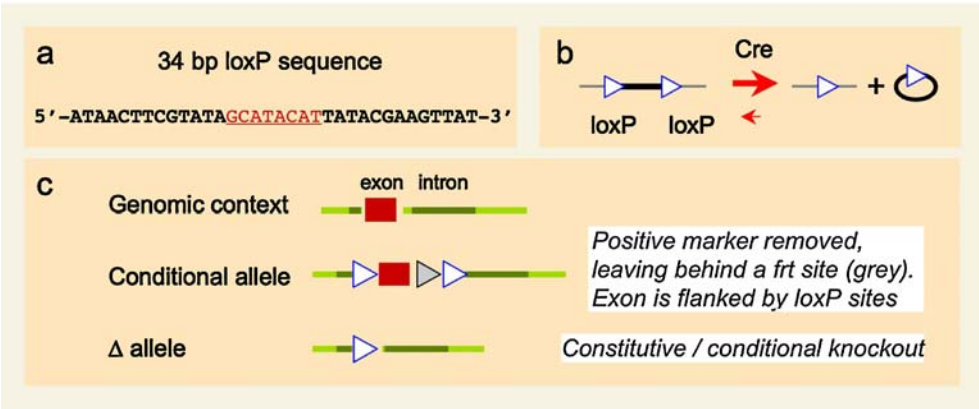


**Fig. 4** Cre/loxP technology to generate and use conditional alleles. **a** The specificity of the Cre/loxP system is given by the 34 bp loxP recognition sequence consisting of two 13-bp palindromic sequences and an 8-bp core sequence. **b** Upon presence of Cre recombinase the loxP-flanked sequence is removed leaving one loxP sequence in the genome. **c** Scheme of the different alleles, wildtype, floxed (conditional) and deleted. In particular, conditional mutants are characterized as carrying floxed alleles (*flox* flanked by loxP sites). This allows elimination of the gene function of interest by transgenic expression of Cre recombinase

development or leads to complex phenotypes. The ability to perform kidney-specific recombination is dependent on the choice of kidney-specific promoters used to drive the expression of Cre recombinase in vivo (Table 2). Such regulatory promoter sequences have to be tested to find out whether they are able to drive the expression of a reporter gene in a nephron-segment-specific manner. Classically, a promoter region of interest is linked to a reporter gene (lacZ, GFP, luciferase) and transgenic mice are generated and analyzed for expression in embryos and various organs. Since Cre expression in such lines in organs beside the kidney has not always been studied extensively, ectopic Cre activity may influence the phenotype of the animal.

We had recently generated transgenic mice that do express Cre recombinase exclusively in the renal proximal tubule [86] using the high-capacity (type2) $Na^+$/glucose cotransporter promoter. Other promoters, like, e.g., the *HoxB7* promoter have been used for targeting Cre recombinase expression to the cortical duct of the kidney but expression has also been reported selectively in the spinal cord and the dorsal root ganglia [113]. Hox genes encode homeodomain-containing proteins that control, e.g., mammalian kidney morphogenesis. During embryonic development, some *Hoxd* genes regulate the metanephric mesenchyme–ureteric bud interactions, and maintenance of structural integrity of tubular epithelia is differentially controlled consistent with their specific expression profile [14]. Mice expressing Cre from the renin promoter are not only useful for kidney-specific recombination, but affect a whole variety of cells given that renin-expressing cells are precursors for certain epithelial and extrarenal cells [95]. And, finally, promoters like, e.g., nephrin affect efficiently the glomeruli as shown for the glomerulus-specific knockout of VEGF-A that led to congenital and acquired renal diseases [17]. The expression of most available Cre recombinase lines is constitutive, but more recently, models have been developed using inducible and ligand-regulated promoter systems to spatially and temporally control Cre recombinase expression in specific renal cell types. Different mouse lines exist which use the $CreER^{T2}$ system, where the Cre recombinase is fused to a mutant human estrogen receptor (ER) and gets only active upon tamoxifen administration [16, 49, 71]. Similarly, a transgenic strain expressing androgen-inducible Cre recombinase has been reported. Here, the transgene is expressed specifically in the renal proximal tubule in male mice, but not expressed in

**Table 2** A survey of Cre transgenic mouse strains targeting the kidney and specific nephron segments

| Cre line | Promoter | Expression | Reference |
|---|---|---|---|
| γGT::Cre | Rat γ-glutamyl transpeptidase II | PT (cort. tubules) | [40] |
| iL1-sglt2::Cre | Mouse sodium–glucose cotransporter 2 | PT | [86] |
| PEPCK::Cre | Rat phosphoenolpyruvate carboxykinase | PT | [32, 79] |
| $GGT::CreER^{T2}$ | Mouse γ-glutamyl transpeptidase II | PT(S3) | [16] |
| KAP2::iCre | Mouse kidney androgen-regulated protein | PT | [53] |
| Ksp1.3::Cre | Mouse kidney-specific cadherin | All, TAL, CD | [96] |
| $KspCad::CreER^{T2}$ | Mouse kidney-specific cadherin | All segments | [48, 49] |
| $Ksp::CreER^{T2}$ | Mouse kidney-specific cadherin | All, CD | [71] |
| THP::Cre | Mouse Tamm–Horsfall protein | TAL | [100] |
| apoE::Cre | Human apolipoprotein (apo) E | PT, DT | [52] |
| AQP2::Cre | Human aquaporin-2 | CD (principal cells) | [67] |
| AQP2::Cre | Mouse aquaporin-2 | CD (principal cells) | [22] |
| AQP2::Cre | Mouse aquaporin-2 | Late CNT, CD (principal cells) | [83] |
| Hoxb7::Cre | Mouse *Hoxb7* | CD | [113] |
| Hoxb7::CreEGFP | Mouse *Hoxb7* | CD | [118] |
| Neph::Cre | Mouse *Nphs1* (nephrin) | Glomeruli, podocytes | [17] |
| NPHS2::Cre | Human podocin (NPHS2) | Glomeruli | [61] |
| $Ren1^d::Cre$ | Mouse renin $1^d$ | Juxtaglomerular cells, afferent arterioles | [95] |
| Pax2::Cre | Mouse *Pax2* | Glomeruli, renal tubules, CD, nephric duct | [68] |
| Pax8::Cre | Mouse *Pax8* | Glomeruli, renal tubules, nepric duct, mesonephros | [7] |
| 11HSD2::iCre | Mouse 11ß-hydroxysteroid dehydrogenase 2 | Kidney (expression in other tissues!) | [66] |
| Pax8::rtTA/LC-1::Cre | Mouse *Pax8* | Renal tubule except glomeruli | [92, 108] |
| Osr2::(Ires)Cre | Mouse Odd-skipped related-2 | Glomeruli | [47] |
| Hnf4a::(Ires)Cre | Mouse hepatocyte nuclear factor α | Cortex (tubules) | [109] |

Some of the strains show Cre expression in other organs as for example gonads (*GGT::CreER*$^{T2}$, *THP::Cre*, *AQP2::Cre*), ureteral epithelium (*Hoxb7::Cre*) or liver and brain (*THP::Cre, PEPCK::Cre*). Please note that Cre recombinase activity in other organs has not always been extensively analyzed and/or documented

*PT* Proximal tubule, *TAL* thick ascending limbs of Henle's loop, *CNT* connecting tubule, *DT* distal tubule, *CD* collecting duct

females unless induced by testosterone [53]. Another efficient and versatile tool for acute and chronic modulation of renal tubular function in transgenic mice has been recently described by Traykova-Brauch and coworkers [108]. They generated *Pax8::rtTA* mice which strongly express the transgene in a highly kidney-specific, uniform and tetracycline-dependent manner. Both inducible and reversible, renal diseases such as polycystic kidney disease, renal fibrosis and renal cancer can be modeled in transgenic mice upon doxycycline application.

A further application of targeting transgenes to the kidney might be the production of human therapeutic proteins: the uromodulin gene promoter has been used to produce recombinant proteins (growth hormone, α1-antitrypsin, erythropoietin) in the urine of transgenic mice [115, 116, 119].

## Mouse models for kidney diseases

This part of the review exemplifies the use of transgenic mouse models for studying renal and electrolyte physiology by targeting gene expression specifically to epithelial cells of the renal tubule. This has been useful, e.g., for studying the intrarenal renin–angiotensin–aldosterone system (RAAS) that is implicated in the development of hypertension, and therefore an important mediator of systemic blood pressure and electrolyte balance. Data obtained from transgenic mice expressing the rat and human angiotensinogen gene [15, 87], the human renin gene [51], and the rat angiotensin II type 1 receptor-associated protein ARAP1 gene [29] to proximal tubules using the kidney-androgen-regulated protein (KAP) promoter underlined the role of the proximal tubule-specific renin–angiotensin system in the regulation of blood pressure and renal function. Further, kidney-specific expression of a cDNA encoding a mutant form of a protein as identified in human is an attractive approach to determine its effects on kidney function. Igarashi et al. expressed a dominant-negative form of the mouse HNF-1β protein that corresponds to the A263insGG mutation as found in human under the control of the kidney-specific *Ksp-cadherin* promoter. The mice developed maturity-onset diabetes of the young-5 (MODY5), with renal cysts and renal failure, similar to the human condition. This kidney-specific transgenic experiment clearly demonstrated that mutations of HNF-1β are sufficient to produce kidney cysts and that the renal cystic disease is intrinsic to the kidney and not secondary to diabetes [31]. To investigate the pathogenic mechanism of the *Pkd1* gain-of-function mutations in the renal tubular epithelium, Trudel and coworkers have produced a transgenic mouse strain that overexpressed the murine *Pkd1* gene using a modified *Pkd1* BAC. These transgenic mice displayed features similar to autosomal dominant polycystic kidney disease (ADPKD) demonstrating that *Pkd1* gain-of-function mutations in the kidney are sufficient to reproduce a renal ADPKD phenotype as found in human [84, 103].

Chung and colleagues used a transgenic approach to address effects on urine concentration and kidney function in vivo in epithelial cells of the renal collecting tubules [46]. Overexpression of a dominant-negative form of the osmotic response element-binding protein (OREBP) under the control of the kidney-specific *Ksp-cadherin* promoter led to polyuria and polydipsia indicating that OREBP is an important regulator of the urine-concentrating mechanism [46]. The knockout of aldose reductase as one of the osmoresponsive genes transcriptionally regulated by TonEBP/OREBP has lost the ability to concentrate urine; in contrast, a knock-in of an aldose reductase transgene (under control of the *Ksp-cadherin* promoter) onto the aldose reductase knockout background revealed an incomplete rescue of the knockout phenotype. The authors concluded that aldose reductase, in addition to its role in osmoregulation, may be essential for the full maturation of the urine-concentrating mechanism [112].

## Kidney-specific knockout models for renal salt and water homeostasis

In the following paragraph, we will give examples of renal tubule epithelial cell-specific knockouts of proteins implicated in salt and water homeostasis. Peroxisome proliferator-activated receptor subtype γ (PPARγ) is the pharmacological target of thiazolidinediones (TZDs) and, within the kidney, is predominantly expressed in the collecting duct. TZDs are widely used antidiabetic drugs, but have well-established side effects such as fluid retention leading to weight gain and occasionally to edema and heart failure [70]. To address the mechanism of TZDs-induced fluid retention, Breyer and colleagues used a mouse strain transgenic for Cre recombinase driven by 14 kb of the human AQP2 5′-flanking region (*AQP2::Cre*) [28, 67]. Yang et al. used *AQP2::Cre* mice using 11 kb of the mouse *AQP2* 5′-flanking region [117]. Using either Cre strain, removal of PPARγ in the collecting duct prevented body weight gain and fluid retention induced by TZDs. These data also underlined a PPARγ-dependent pathway of sodium transport in the collecting duct, with TZDs-induced fluid retention. Concerning renal regulation of water balance and urinary concentrating process, mice lacking AQP2 expression in CD but with sustained AQP2 expression in CNT using *HoxB7::Cre* mice were generated [82]. Mutant mice survived to adulthood but exhibited severe polyuria and deterioration of urinary concentrating ability after water deprivation. The model demonstrated that AQP2 expression in CNT is sufficient for postnatal survival and in CD is essential for the regulation of body water

homeostasis. To better understand the role of the medullary endothelin-1 (ET-1) system in regulating renal salt and water excretion and systemic blood pressure, Kohan and colleagues have engineered several mouse models using Cre/loxP technology and *AQP2::Cre* transgenic mice expressing Cre recombinase in the collecting duct. Mice with CD-specific downregulation of ET-1 are hypertensive and have impaired sodium excretion in response to sodium loading [1]. These mice also have decreased ability to excrete an acute water load and enhanced sensitivity to vasopressin [19]. Moreover, in these animals, urinary prostaglandin E2 (PGE2) excretion was increased which partly compensates for loss of ET-1 [21]. In addition, the role of ET receptors was also assessed by disrupting $ET_A$ or $ET_B$ receptors specifically in CD. $ET_B$ loss-of-function in CD results in hypertension and reduced $Na^+$ excretion after an acute $Na^+$ load leading to the conclusion that $ET_B$ receptors partially mediate ET-1 functions as a natriuretic and antihypertensive factor [20]. In a separate study, the authors highlighted the role of CD $ET_A$ receptors in reducing ET-1 inhibition of vasopressin signaling [22]. Recently, the role for nitric oxide (NO) pathway in CD-derived ET-1-induced natriuresis, diuresis, and hypotension was unveiled [91]. With regard to regulation of urine concentration mechanism, the role of the Pax transactivation-domain interacting protein (PTIP) in the inner renal medulla was studied by tissue-specific gene deletion using *Ksp1.3::Cre* mice, demonstrating the functional importance of PTIP in urine-concentrating ability by modulating arginine vasopressin receptor 2 and AQP2 expression in the renal collecting ducts [44].

To assess the role of a proximal tubular endocytic receptor, megalin, in calcium and phosphate homeostasis, two reports analyzed kidney-specific megalin knockout mice generated by conditional gene targeting using *apoE::Cre* mice. Renal specific deletion of megalin resulted in plasma vitamin D deficiency, hypocalcemia and bone disease [52]. Proximal tubular type IIa sodium phosphate cotransporter (NaPi-IIa) handling is also affected in this model and disruption of endocytosis resulted in reduced phosphaturia [3]. These two studies highlighted an essential role of the megalin receptor pathway in calcium and phosphate homeostasis. Tiwari et al. removed the insulin receptor specifically in renal epithelial cells in the mouse revealing its role in the kidney for sodium balance and blood pressure [107]. To examine the role of the mineralocorticoid receptor (MR) in sodium homeostasis, a new *AQP2::Cre* mouse strain was used to inactivate MR in collecting duct and late connecting tubule [83]. The mutant mice exhibited normal renal sodium excretion associated with elevated aldosterone levels on a standard diet. However, this compensation by the late distal convoluted tubule and early connecting tubule fails when challenged with a low-salt diet leading to loss of body weight associated with increased renal sodium and water excretion.

Lastly, we will discuss the genetic dissection of the highly amiloride-sensitive epithelial sodium channel ENaC along the nephron as an example of a constitutively as well as conditionally targeted sodium transporting protein in the kidney. Over the past years, we have generated an allelic series of mutations at the ENaC (*Scnn1*) gene loci showing that any modified expression the ENaC subunits may cause a kidney disease [4, 36, 39, 74, 76, 111]. Constitutive gene inactivation of all three subunits revealed that the absolute ENaC expression is essential for survival [4, 35, 59]. Removal of the αENaC subunit (*Scnn1a*) resulted in completely abolished ENaC activity, whereas inactivation of the ßENaC (*Scnn1b*) and γENaC (*Scnn1g*) subunit led to reduced ENaC activity. Mice without ßENaC (*Scnn1b*) and γENaC (*Scnn1g*) develop hyperkalemia and die soon after birth [4]. Failure to thrive and lethargy are associated with urinary $Na^+$ wasting, $K^+$ retention, and increased plasma aldosterone concentrations (for review, see [39]). Mutations that result in hypofunction in the kidney are expected to induce a salt wasting syndrome similar to type 1 pseudohypoaldosteronism (see, for review [33]). Various mouse lines have been generated in which the ENaC activity ranges from hypoactive channels (5–15% of total ENaC activity) to hyperactive channels (>150% of total ENaC activity; for review, see [37]. Reduced ENaC activity in mice bearing ENaC mutations led to clinical symptoms similar to PHA-1 (pseudohypoaldosteronism type 1) ranging from mild (e.g., mutation in the ßENaC gene locus [74]) to severe phenotype (e.g., γENaC knockout mice [4]). We further introduced one of the classical Liddle mutations (R566STOP) into the mouse ßENaC (*Scnn1b*) gene locus, thereby generating mice, which reproduce to a large extent the clinical symptoms of Liddle patients [76]. These mice present an impaired ENaC internalization, and exhibit ENaC-mediated transport features that are consistent with an overall increased ENaC activity [75]. Interestingly, mineralocorticoid-mediated up-regulation of ENaC expression and function is still maintained in these mice which show a remarkable high sensitivity to aldosterone in vivo [13, 75]. Renal cells from these mice exhibit hyperactive apical vasopressin-regulated CFTR $Cl^-$ conductance [12] that could contribute to the enhanced NaCl reabsorption observed in the distal nephron of patients with Liddle's syndrome (for review, see [34]). Finally, we used conditional gene targeting of ENaC to genetically dissect the aldosterone-induced ENaC-mediated sodium reabsorption along the nephron. When a floxed allele for the αENaC subunit ($Scnn1a^{lox/lox}$ mice) [38] was crossed with the *HoxB7::Cre* line this resulted in a complete abolishment of ENaC function in the cortical collecting duct (CCD), but not in the early segments of the aldosterone-sensitive distal

nephron (ASDN), the late distal convoluted tubule (DCT) and connecting tubule (CNT)[85]. Surprisingly, we found that ENaC expression in the CCD is not a prerequisite for normal sodium and potassium balance. We further concluded that aldosterone-regulated ENaC activity might occur more proximal in the early ASDN. Further tools to dissect ENaC function along the nephron include the *AQP2::Cre* mice that have been recently used to knockout the mineralocorticoid MR receptor in the CNT and CCD (Christensen et al., manuscript in preparation; [83]) and the *Pax8::rtTA/LC1::Cre* double transgenic line [108], that should eliminate ENaC function in all kidney cells with exception of glomeruli when bred to the floxed αENaC mice (*Scnn1a*$^{flox}$). Elimination of ENaC in the whole kidney will certainly indicate to which extent the kidney contributes to the whole net sodium homeostasis of the body. The ultimate proof of the aldosterone-dependent sodium reabsorption will be the specific inactivation of ENaC within the DCT2 and CNT by crossing the floxed ENaC mice, e.g., with mice that express the Cre recombinase under the control of the TRPV5 promoter [77]. The identification of the exact nephron segment involved in aldosterone-dependent sodium reabsorption will help to develop a more refined treatment for hypertension.

## Conclusion

In summary, renal cell-specific expression and renal cell-specific knockout approaches are effective tools for demonstrating in vivo importance of genes in ion and water homeostasis in the adult kidney. With a growing list of nephron-segment-specific promoters and nephron-segment-specific Cre mouse lines, any gene of interest can now be expressed or removed within the nephron to study renal physiology and pathophysiology. Such studies will finally lead to more refined drugs in kidney disease.

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